

What is claimed is:

1. A method of modifying a mismatched duplex DNA, said method comprising contacting an MSH dimer and said mismatched duplex DNA in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein  
5 the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said MSH dimer associates with the mismatched region of said mismatched duplex DNA, thereby modifying said mismatched duplex DNA.
2. The method of claim 1, wherein said MSH dimer is selected from the group  
10 consisting of a prokaryotic MSH homodimer, a prokaryotic MSH heterodimer, a eukaryotic MSH homodimer, and a eukaryotic MSH heterodimer.
3. The method of claim 2, wherein said MSH dimer is a homodimer of a MutS  
15 homolog selected from the group consisting of a human MutS homolog, a murine MutS homolog, a rat MutS homolog, a Drosophila MutS homolog, a yeast MutS homolog, and a Saccharomyces cerevisiae MutS homolog.
4. The method of claim 2, wherein said eukaryotic MSH homodimer is an  
20 MSH2 homodimer.
5. The method of claim 2, wherein said eukaryotic MSH heterodimer comprises  
MutS homologs independently selected from the group consisting of an MSH2 protein, an MSH3 protein, an MSH4 protein, an MSH5 protein, and an MSH6 protein.
- 25 6. The method of claim 5, wherein said MSH dimer is selected from the group consisting of an MSH2:MSH3 heterodimer, an MSH2:MSH6 heterodimer, and an MSH4:MSH5 heterodimer.
- 30 7. The method of claim 2, wherein said prokaryotic MSH dimer is a homodimer of Escherichia coli MutS.

8. The method of claim 1, wherein said MSH dimer is substantially purified.

5 9. The method of claim 1, wherein the concentration of ATP in said binding solution is less than about 0.3 micromolar.

10 10. The method of claim 9, wherein said binding solution is substantially free of ATP.

11. The method of claim 1, wherein at least one of said MSH dimer and said mismatched duplex DNA is bound to a support.

12. The method of claim 1, wherein said mismatched duplex DNA has at least one free end.

13. The method of claim 1, wherein said mismatched duplex DNA comprises a DNA strand generated by reverse transcription of mRNA obtained from an organism.

14. The method of claim 1, wherein said mismatched duplex DNA comprises a first DNA strand having a reference nucleotide sequence and a second DNA strand selected from the group consisting of a DNA strand obtained from an organism, a DNA strand obtained by amplification of at least a portion of a polynucleotide obtained from an organism, a DNA strand obtained by cleavage of a polynucleotide obtained from an organism, and a DNA strand obtained by reverse transcription of a polynucleotide obtained from an organism.

15. The method of claim 14, wherein said second DNA strand comprises at least a portion of a gene associated with a cancer in said organism.

16. The method of claim 15, wherein said organism is a human and wherein said gene is selected from the group consisting of an oncogene and a tumor suppressor gene.

17. The method of claim 16, wherein said gene is selected from the group consisting of abl, akt2, apc, bcl2alpha, bcl2beta, bcl3, bcr, brca1, brca2, cbl, ccnd1, cdk4, crk-II, csf1r/fms, dbl, dcc, dpc4/smad4, e-cad, e2f1/rbap, egfr/erbB-1, elk1, elk3, eph, erg, ets1, ets2, fer, fgr/src2, fli1/erbB2, fos, fps/fes, fra1, fra2, fyn, hck, hek, her2/erbB-2/neu, her3/erbB-3, her4/erbB-4, hras1, hst2, hstf1, ink4a, ink4b, int2/fgf3, jun, junb, jund, kip2, kit, kras2a, kras2b, lck, lyn, mas, max, mcc, met, mlh1, mos, msh2, msh3, msh6, myb, myba, mybb, myc, mycl1, mycn, nf1, nf2, nras, p53, pdgfb, pim1, pms1, pms2, ptc, pten, raf1, rb1, rel, ret, ros1, ski, src1, tal1, tgfb2, thra1, thrb, tiam1, trk, vav, vhl, waf1, wnt1, wnt2, wt1, and yes1.

18. The method of claim 17, wherein said cancer is hereditary non-polyposis colon cancer and said gene is selected from the group consisting of mlh1, msh2, msh3, msh6, pms1, and pms2.

19. The method of claim 18, wherein said cancer is selected from the group consisting of a leukemia, a lymphoma, a meningioma, a mixed tumor of a salivary gland, an adenoma, a carcinoma, an adenocarcinoma, a sarcoma, a dysgerminoma, a retinoblastoma, a Wilms' tumor, a neuroblastoma, a melanoma, and a mesothelioma.

20. The method of claim 1, wherein said mismatched duplex DNA and said MSH dimer are contacted in the presence of at least one non-mismatched duplex DNA.

21. The method of claim 20, further comprising separating said MSH dimer from said non-mismatched duplex DNA after contacting said mismatched duplex DNA and said MSH dimer.

22. The method of claim 21, further comprising dissociating said mismatched duplex DNA and said MSH dimer after separating said MSH dimer from said non-mismatched duplex DNA and thereafter amplifying said mismatched duplex DNA.

23. The method of claim 22, wherein said MSH dimer is bound to a support prior to separating said non-mismatched duplex DNA from said MSH dimer.

5 24. The method of claim 23, wherein said non-mismatched duplex DNA is separated from said MSH dimer in the presence of a separating solution, wherein said separating solution is substantially free of ATP.

10 25. The method of claim 24, further comprising releasing said mismatched duplex DNA from said MSH dimer after separating said non-mismatched duplex DNA from said MSH dimer.

15 26. The method of claim 25, wherein said mismatched duplex DNA has at least one free end and is released from said MSH dimer by contacting said MSH dimer with a releasing solution selected from the group consisting of a solution comprising ATP and  $Mg^{2+}$  ions, a solution comprising ATP and a magnesium-chelating agent, a solution comprising high salt, a solution comprising a gamma-modified ATP analog and  $Mg^{2+}$  ions, and a solution comprising a gamma-hydrolysis-resistant ATP analog and  $Mg^{2+}$  ions.

20 27. The method of claim 26, wherein said releasing solution comprises ATP and  $Mg^{2+}$  ions.

25 28. The method of claim 25, wherein said mismatched duplex DNA does not have a free end and is released from said MSH dimer by contacting said MSH dimer with a releasing solution selected from the group consisting of a solution comprising a magnesium-chelating agent, a solution comprising high salt, a solution comprising a double-stranded DNA cleaving enzyme, ATP and  $Mg^{2+}$  ions, a solution comprising a double-stranded DNA cleaving enzyme, a gamma-modified ATP analog, and  $Mg^{2+}$  ions, and a solution comprising a double-stranded DNA cleaving enzyme, a gamma-hydrolysis-resistant ATP analog, and  $Mg^{2+}$  ions.

29. The method of claim 21, further comprising contacting said MSH dimer with a MutL homolog after contacting said mismatched DNA and said MSH dimer.

30. The method of claim 1, further comprising detecting association of said MSH dimer with said mismatched duplex DNA.

31. The method of claim 30, wherein association of said MSH dimer with said mismatched duplex DNA is detected using an assay selected from the group consisting of a gel mobility shift assay, a filter binding assay, an immunological assay, a sedimentation centrifugation assay, a spectroscopic assay, an optical affinity assay, a DNA footprint assay, and a nucleolytic cleavage protection assay.

32. The method of claim 1, wherein said duplex DNA does not have a free end.

33. The method of claim 32, wherein said MSH dimer is present in molar excess with respect to said mismatched duplex DNA, whereby an average of more than one said MSH dimer associates with one molecule of said mismatched duplex DNA.

34. A method of modifying a mismatched duplex DNA which does not have a free end, said method comprising contacting said mismatched duplex DNA and an MSH dimer having ADP bound thereto in the presence of a binding solution, wherein the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said homolog associates with the mismatched region of said mismatched duplex DNA, thereby modifying said mismatched duplex DNA.

35. A method of segregating a mismatched duplex DNA from a population of DNA molecules, said method comprising

contacting an MSH dimer and said population in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein the

concentration of ATP in said binding solution is less than about 3 micromolar, whereby said MSH dimer associates with said duplex DNA; and

5 segregating said MSH dimer from said population, whereby said mismatched duplex DNA is segregated from said population.

36. A method of detecting a difference between a sample nucleotide sequence and a reference nucleotide sequence, said method comprising

- 10 a) annealing a first DNA strand and a second DNA strand to form a duplex DNA,
- i) wherein said first DNA strand has said sample nucleotide sequence
  - ii) wherein said second DNA strand has a nucleotide sequence which is complementary to said reference nucleotide sequence, and
  - 15 iii) whereby if there is a difference between said sample nucleotide sequence and said reference nucleotide sequence then said duplex DNA is a mismatched duplex DNA;
- b) thereafter contacting said duplex DNA and an MSH dimer in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said
- 20 MSH dimer associates with said duplex DNA if said duplex DNA is a mismatched duplex DNA; and
- c) determining whether said MSH dimer is associated with said duplex DNA molecule,
- 25 whereby association of said MSH dimer with said duplex DNA molecule is an indication that there is a difference between said sample nucleotide sequence and said reference nucleotide sequence.

37. A kit for separating a mismatched duplex DNA from non-mismatched duplex DNAs, said kit comprising

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at least two MutS homologs;  
a linker for binding said at least one of said MutS homologs to a support; and  
an additional reagent selected from the group consisting of a nucleotide and a releasing  
solution, wherein said nucleotide is selected from the group consisting of ADP and ATP, and  
5 wherein said releasing solution comprises  $Mg^{2+}$  and a compound selected from the group  
consisting of ATP, a gamma-modified ATP analog, and a gamma-hydrolysis-resistant ATP  
analog.

38. A method of determining whether a mammal is predisposed for  
10 carcinogenesis, said method comprising

- a) annealing a first DNA strand and a second DNA strand to form a duplex DNA,  
i) wherein said first DNA strand has the nucleotide sequence of at least a portion of  
a gene selected from the group consisting of an oncogene and a tumor suppressor gene  
15 of said mammal, and  
ii) wherein said second DNA strand has a nucleotide sequence which is  
complementary to the consensus nucleotide sequence of said region,  
iii) whereby if there is a sequence difference between said first DNA strand and said  
second DNA strand then said duplex DNA is a mismatched duplex DNA;

- 20 b) thereafter contacting said duplex DNA and an MSH dimer in the presence of a binding  
solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein  
the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said  
MSH dimer associates with said duplex DNA if said duplex DNA is a mismatched duplex  
25 DNA; and

- c) determining whether said MSH dimer is associated with said duplex DNA,

whereby association of said MSH dimer with said duplex DNA is an indication that said  
30 mammal is predisposed for carcinogenesis.

39. A method of fractionating a population of duplex DNAs, said method comprising

5 a) contacting said population with an MSH dimer in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said MSH dimer associates with at least one mismatched duplex DNA in said population; and

10 b) segregating said MSH dimer from said population of duplex DNAs,

whereby said mismatched duplex DNA is segregated from said population of duplex DNAs, whereby said population is fractionated.

15 40. A method of selectively amplifying at least one mismatched duplex DNA of a population of duplex DNAs, said method comprising

contacting said population with an MSH dimer in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein the  
20 concentration of ATP in said binding solution is less than about 3 micromolar, whereby said MSH dimer associates with said mismatched duplex DNA,

thereafter segregating said MSH dimer from said population of duplex DNAs, whereby said mismatched duplex DNA is segregated from said population of duplex DNAs, and

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thereafter amplifying said mismatched duplex DNA,  
whereby said mismatched duplex DNA is selectively amplified.



41. A method of determining whether the nucleotide sequence of a first copy of a genomic sequence differs from the nucleotide sequence of a second copy of said genomic sequence, said method comprising

5        amplifying a region of each of said first copy and said second copy of said genomic sequence to yield amplified first copies and amplified second copies;

      mixing and denaturing said amplified first copies and said amplified second copies to form a first mixture;

10        thereafter annealing the nucleic acids in said first mixture to form a second mixture comprising duplex DNAs, whereby if said the nucleotide sequence of first copy and the nucleotide sequence of said second copy of said genomic sequence differ then at least some of said duplex DNAs are mismatched duplex DNAs;

15        thereafter contacting said second mixture with an MSH dimer in the presence of a binding solution comprising a nucleotide selected from the group consisting of ADP and ATP, wherein the concentration of ATP in said binding solution is less than about 3 micromolar, whereby said MSH dimer associates with said mismatched duplex DNAs; and

20        determining whether said MSH dimer is associated with at least some of said duplex DNAs,

25        whereby association of said MSH dimer with said at least some of said duplex DNAs is an indication that the nucleotide sequence of said first copy of said genomic sequence differs from the nucleotide sequence of said second copy of said genomic sequence.

42. A composition for segregating a mismatched duplex DNA from a population of duplex DNAs, said composition comprising an MSH heterodimer bound to a support.

43. A kit for screening a genomic region for a nucleotide sequence which differs from a reference nucleotide sequence, said kit comprising  
a pair of primers complementary to the ends of said region for amplifying said region;  
a DNA strand having said reference nucleotide sequence; and  
at least two MutS homologs.

44. A nonhuman mammal which is nullizygous for both Msh2 and p53, wherein said mammal does not express Msh2 or p53, and wherein said mammal exhibits a phenotype selected from the group consisting of inappropriate fetal apoptosis and a predisposition for carcinogenesis.

45. A method of making a nonhuman mammal which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a phenotype selected from the group consisting of a predisposition for inappropriate fetal apoptosis and a predisposition for carcinogenesis, said method comprising mating

a) a first parent mammal comprising at least one null allele of Msh2 and at least one null allele of p53 and

b) a second parent mammal comprising at least one null allele of Msh2 and at least one null allele of p53,

whereby a nonhuman mammal is generated which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a phenotype selected from the group consisting of inappropriate fetal apoptosis and a predisposition for carcinogenesis.

46. A method of determining whether a compound affects tumorigenesis in mammals, said method comprising

administering said compound to a first nonhuman mammal which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a predisposition for carcinogenesis, and

5 comparing tumor incidence in said first nonhuman mammal with tumor incidence in a second nonhuman mammal of the same type which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, exhibits a predisposition for carcinogenesis, and to which said compound is not administered,

10 whereby a difference in tumor incidence in said first transgenic mammal compared with tumor incidence in said second transgenic mammal is an indication that said compound affects tumorigenesis in mammals.

47. A method of determining whether a compound affects a biological  
15 phenomenon in mammals, said phenomenon selected from the group consisting of apoptosis, aging, and fetal development, said method comprising

administering said compound in utero to a first nonhuman mammalian embryo which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a  
20 predisposition for inappropriate fetal apoptosis, and

comparing the development of said first nonhuman mammalian embryo with the development of a second nonhuman mammalian embryo of the same type which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, exhibits a  
25 predisposition for inappropriate fetal apoptosis, and to which said compound is not administered,

whereby a difference in the development of said first nonhuman mammalian embryo compared with the development of said second nonhuman mammalian embryo is an indication that said  
30 compound affects said biological phenomenon in mammals.

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5 48. A cell line which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a phenotype selected from the group consisting of a predisposition for carcinogenesis and a predisposition for apoptosis, wherein said cell line is made by culturing a cell obtained from the nonhuman mammal of claim 56.

49. A method of determining whether a composition affects expression of a gene selected from the group consisting of the p53 gene and a gene encoding a MutS homolog, said method comprising

10 administering said composition to a first non-human mammal which is nullizygous for one of said p53 gene and said gene encoding a MutS homolog;

15 comparing a phenotype of said non-human mammal with said phenotype of a second non-human mammal of the same type which is not nullizygous for said one of said p53 gene and said gene encoding a MutS homolog, wherein said phenotype is selected from the group consisting of inappropriate fetal apoptosis and a predisposition for carcinogenesis;

20 whereby a difference between said phenotype of said first non-human mammal and said phenotype of said second non-human mammal is an indication that said composition affects expression of the other of said p53 gene and said gene encoding a MutS homolog.

25 50. A method of determining whether a composition affects expression of a gene selected from the group consisting of the p53 gene and a gene encoding a MutS homolog, said method comprising

30 administering said composition to a first cell derived from a non-human mammal which is nullizygous for one of said p53 gene and said gene encoding a MutS homolog;

5 comparing a phenotype of said first cell with said phenotype of a second cell derived from a non-human mammal of the same type which is not nullizygous for said one of said p53 gene and said gene encoding a MutS homolog, wherein said phenotype is selected from the group consisting of inappropriate fetal apoptosis and a predisposition for carcinogenesis;

whereby a difference between said phenotype of said first cell and said phenotype of said second cell is an indication that said composition affects expression of the other of said p53 gene and said gene encoding a MutS homolog.

10 51. A composition comprising a human MutS homolog fragment, wherein said fragment comprises a MutS homolog interaction region.

15 52. A method of inhibiting association of a first human MutS homolog and a second human MutS homolog, said method comprising contacting at least one of said first human MutS homolog and said second human MutS homolog with a human MutS homolog fragment comprising a MutS homolog interaction region, whereby association of said first human MutS homolog and said second human MutS homolog is inhibited.

20 53. A composition comprising substantially purified hMSH5.

54. A composition comprising an isolated nucleic acid encoding hMSH5.

25 55. A method of modifying a mismatched duplex DNA, said method comprising contacting an MSH dimer and said mismatched duplex DNA in the presence of a binding solution comprising ADP, wherein the concentration of ADP in said binding solution is at least about ten times the concentration of ATP, if ATP is present in said binding solution, whereby said MSH dimer associates with the mismatched region of said mismatched duplex DNA, thereby modifying said mismatched duplex DNA.